Stem Cells and Their Role in Renal Ischaemia Reperfusion Injury

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Key Words

Stem cells · Ischaemia reperfusion injury · Acute renal injury · Transplantation

Abstract

Background: Ischaemia-reperfusion injury (IRI) remains one of the leading causes of acute kidney injury (AKI). IRI is an underlying multifactorial pathophysiological process which affects the outcome in both native and transplanted patients. The high morbidity and mortality associated with IRI/AKI and disappointing results from current available clinical therapeutic approaches prompt further research. Stem cells (SC) are undifferentiated cells that can undergo both renewal and differentiation into one or more cell types which can possibly ameliorate IRI. **Aim:** To carry out a detailed literature analysis and construct a comprehensive literature review addressing the role of SC in AKI secondary to IRI. **Methods:** Evidence favouring the role of SC in renal IRI and evidence showing no benefits of SC in renal IRI are the two main aspects to be studied. The search strategy was based on an extensive search addressing MESH terms and free text terms. **Results:** The majority of studies in the field of renal IRI and stem cell therapy show substantial benefits. **Conclusions:** Studies were mostly conducted in small animal models, thus underscoring the need for further pre-clinical studies in larg-

er animal models, and results should be taken with caution. SC therapy may be promising though controversy exists in the exact mechanism. Thorough scientific exploration is required to assess mechanism, safety profile, reproducibility and methods to monitor administered SC.

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Introduction

Ischaemia-reperfusion injury (IRI) remains one of the leading causes of acute kidney injury (AKI) in both native and transplanted kidneys [1, 2]. IRI is an underlying multifactorial pathophysiological process which affects the outcome in many clinical situations, in particular transplantation [3, 4]. Transplantation, the therapy for ESRD, is associated with several deficiencies: lack of suitable organs, immunosuppression, surgical morbidity and poorer outcomes associated with use of marginal organs [5] [extended-criteria donors (ECDs) and donors after circulatory death (DCDs)] and patients waiting longer on the waiting list [6–8]. The process of IRI is accelerated in recipients receiving organs from ECDs and DCDs. This reflects in increased rates of delayed graft function and primary non-function as a consequence of the associated ischaemia especially the prolonged warm ischaemic in-

sult sustained during organ retrieval and added cold storage time [4, 9–10]. In the native kidney situation, AKI affects 7–10% of all hospitalised patients and can be associated with a mortality rate of 35–40% for inpatients and 75% for patients with sepsis-associated renal failure [11]. The current management for AKI is non-specific and associated with limited supportive care; thus, the need for more novel approaches is warranted [12].

The pathophysiological process of AKI following IRI leads to functional and structural changes that are centred around the proximal tubule cells and endothelium [13]. The pathological events involve disruption of the actin cytoskeleton, alteration in adenine nucleotide metabolism and intracellular calcium, loss of cell-cell/cell-ECM attachments, generation of reactive oxygen molecules and inflammatory responses following activation of endothelium leading to recruitment and infiltration of effector cells and upregulation of apoptosis [14–19]. The kidney can recover remarkably by subsequent events involving cell de-differentiation and proliferation. This is achieved by participation of integrins and cell adhesion molecules which are regulators of migration, differentiation and proliferation. The local release of growth factors like hepatic growth factor (HGF), insulin-like growth factor (IGF), chemokines and cytokines coordinate this cell proliferation [19–21]. Repolarisation and differentiation can be brought about by other factors like neural cell adhesion molecule and osteopontin [13].

Stem cells (SC) are undifferentiated cells defined by their ability for self-renewal and the capacity for multilineage differentiation [22–24]. The mechanisms of action for stem cell therapy are under debate. Current thoughts as to how they might ameliorate renal IRI [25–31] are as follows: (1) transdifferentiation/de-differentiation/homing of SC; (2) paracrine or endocrine effects of grafted SC; (3) role of resident stem/progenitor cell expansion and proliferation.

Based on their expression of cell surface markers, transcription factors and cytokines, a stem cell can be categorised as being either cytological or functional [23].

Bone marrow-derived SC (BMSC) can be classified as follows: (1) haematopoietic SC (HSC), which give rise to all differentiated blood cells [31]; (2) mesenchymal SC (MSC)/marrow stromal cells which differentiate into MSC like chondrocytes, osteocytes and adipocytes [23, 24, 31].

Additionally, adult SC of various types have been identified in tissues like intestine, adipose, skeletal muscle, myocardium and renal tissue [32, 33]. Adult SC from skeletal muscle contain unipotent satellite cells which

fuse and differentiate into multinucleated myotubes [34, 35], and have been shown to give rise to cells of different lineages [36, 37]. These various SC have been shown in various experimental studies to alter the various IRI responses described above and possibly ameliorate the functional parameters by accelerating regeneration of proximal tubular cells. SC can lead to inhibition of release of various proinflammatory cytokines and secrete various trophic growth factors that promote angiogenesis, mitogenesis and proliferation while reducing apoptosis [38], attenuate inflammation and initiate repair [39].

The emerging field of regenerative medicine is progressing rapidly and is supported by a large number of studies demonstrating the capacity of SC to substitute for the damaged or lost differentiated cells in various organs and tissues [40–43]. There is considerable debate as to whether the effect of these cells is due to transdifferentiation or their paracrine properties [25–31, 44–49]. SC therapy may have the potential to be developed into the novel therapeutic approach to address AKI.

Aims

The aim of this review was to carry out a detailed literature analysis and construct a comprehensive literature review addressing the role of SC in AKI secondary to renal IRI. This in-depth document will thus address in detail the following two main aspects: (1) evidence favouring the role of SC in renal IRI; (2) evidence showing no benefits of SC in renal IRI.

Methods and Search Strategy

An extensive search of MEDLINE (PubMed – 1966–2012), The Cochrane Central Register of Controlled Trials, EMBASE (1974–2010), The Database of Abstracts of Reviews of Effects, Health Technology Assessment Database was performed to identify relevant studies (fig. 1).

The search strategy was based on the following terms: MESH terms – Reperfusion Injury, Acute Kidney Injury, Stem Cells, Adult Stem Cells (ASC), Embryonic Stem Cells (ESC), Mesenchymal Stem Cells, Endothelial Cells (ECs), Hematopoietic Stem Cells, Tissue Therapy, Kidney Transplantation, Delayed Graft Function, Humans, Cell Transplantation; free text terms – Renal Ischaemia Reperfusion Injury, Adipose Tissue-Derived Stem Cells, Adipose Tissue-Derived Stem and Regenerative Cells (ADRC), Endothelial Progenitor Cells (EPCs), Neural Precursor Cells (NPC), Bone Marrow-Derived Stem Cells, Bone Marrow Mesenchymal Stem Cells (BM-MSC), Bone Marrow-Derived Endothelial Progenitor Cells, Granulation Tissue Stem Cells (GTSC), Autologous Mesenchymal Stem Cells, Cell Therapy, Transdifferentiation of Stem Cells, Paracrine Action of Stem Cells.

The search had no date restrictions and included experimental, clinical, review and editorial articles in the English literature. The search generated 175 articles, out of which 53 were deemed not relevant. The 122 referenced documents include 70 experimental, 13 clinical, 36 reviews and 3 editorial articles.

Evidence Favouring SC in Renal IRI

Transdifferentiation/De-Differentiation/Homing of SC (table 1)

Brodsky et al. [50] observed that during renal IRI, endothelial cells were severely damaged, and transplantation of adult human endothelial cells into an athymic nude rat which was subjected to renal ischaemia resulted in protection of kidney against injury and dysfunction. The group in 2004 showed that skeletal muscle provides a rich source of skeletal muscle-derived SC and delineated protocols for their in vitro expansion and differentiation along the endothelial lineage, thereby demonstrating engraftment of transplanted cells into renal microvasculature [32]. Lin et al. [31] in a ROSA26 mouse model showed that HSC were able to differentiate into renal tubular cells and augment renal function after renal IRI. The published data showed that on polymerase chain reaction analysis, the presence of male-specific Sry gene and Y chromosome fluorescent in situ hybridisation confirmed the presence of male-derived cells in kidneys of female recipients, thus supporting the homing/de-differentiation hypothesis. Similarly, Jiang et al. [51] in the rat model showed improvement of IRI, and the beneficial effects were due to differentiation of bone marrow MSCs (BM-MSC) into the phenotype of damaged tissue cells where they were able to demonstrate homing of BM-MSC with a high engraftment level of nearly 20%.

Paracrine/Endocrine Effects of Grafted SC (table 1)

In contrast, Togel et al. [23, 52] demonstrated in a rat IRI model that considerable significant renoprotection was obtained with multipotent MSCs, though none of the administered MSCs had differentiated into tubular or endothelial cell phenotypes. The beneficial effects they concluded were primarily mediated via a complex paracrine action. This was through amelioration of inflammatory, vascular and apoptotic/necrotic manifestations of renal IRI. A significant reduction of proinflammatory cytokines like interleukin-1- β (IL-1 β), tumour necrosis factor- α (TNF- α), interferon- γ (IFN- γ) and inducible nitric oxide synthase, as well as an upregulation of anti-inflammatory cytokines like IL-10, basic fibroblast growth factor, transforming growth factor- α and B-cell lymphoma

Fig. 1. PRISMA flow diagram.

(2Bcl-2) was demonstrated. Similarly, Semedo et al. [53] demonstrated an anti-inflammatory pattern in MSC-treated rats, where higher levels of anti-inflammatory cytokines (IL-4) and reduced levels of proinflammatory cytokine IL-1 β were demonstrated. Recently, Patel et al. [54] demonstrated in an IRI rat model the beneficial paracrine role of GTSC. They showed that GTSC treatment led to a 2- to 8-fold increased expression of growth factors (fibroblast growth factor 2, HGF, IGF-1, vascular endothelial growth factor, VEGF) and anti-inflammatory factors (IL-4, IL-10). The authors thereby concluded that foreign body-induced GTSC ameliorate IRI in rats, and may form an important source of SC because of easier accessibility and abundance compared to SC from bone marrow [54].

IRI leading to renal tissue damage is associated with Th (T helper)-1-induced immune responses. Thus, MSC have an ability to deviate the Th1/Th2 response which is seen following IRI, supporting the paracrine function of SC [53]. Semedo et al. demonstrated that the immunomodulatory effects of MSC occur at a very early time point, thus changing the inflammatory profile towards a

Table 1. Studies highlighting benefits of SC therapy in small animal models

Reference	SC source	Animal	Mode of action	Features
Brodsky et al. [50]	human umbilical vein endothelial cells	rat	homing of SC (H)	↑ renal functional protection of ischaemic kidney
Lin et al. [31]	mouse HSC	mouse	H	↑ renal function ↑ β -galactosidase-positive cells ↑ tubular cell differentiation
Arriero et al. [32]	mouse skeletal muscle-derived SC	mouse	H	↑ short-term renal function ↑ differentiation into endothelial cell lineage
Jiang et al. [51]	rat BM-MSC	rat	H	↑ engraftment (20%) of BM-MSC seen by Y chromosome in situ hybridisation ↑ improvement in functional and histological parameters
Morigi et al. [81]	mouse BM-MSC	mouse	paracrine and endocrine effects (P/E)	↑ proliferation ↑ morphological recovery ↑ renal function
Tögel et al. [23]	rat BM-MSC	rat	P/E	↑ renal function ↓ apoptosis and renal injury ↓ IL-1 β , TNF- α , IFN- γ , iNOS ↑ IL-10, bFGF, TGF- α , Bcl-2
Tögel et al. [52]	rat BM-MSC	rat	P/E	↓ apoptosis and inflammation ↓ IL-1 β , TNF- α , IFN- γ , iNOS
Imberti et al. [120]	mouse BM-MSC	mouse	P/E	↑ renal function and ↓ tubular injury ↑ VEGF, HGF, IGF-1
Bi et al. [84]	mouse BM-MSC	mouse	P/E	↑ renal function ↑ tubular cell proliferation ↓ tubular cell atrophy
Semedo et al. [53]	rat BM-MSC	rat	P/E	↓ serum creatinine and plasma urea ↑ PCNA nuclei in MSC-treated kidney ↑ IL-4, ↓ IL-1 β
Semedo et al. [13]	rat BM-MSC	rat	P/E	↑ renal function and proliferation ↓ apoptosis and renal injury ↑ Bcl-2/Bad ratio change to Thy-2 inflammatory profile ↓ IL-1 β , IL-6, TNF- α ↑ IL-4, IL-10
Westenfelder et al. [115]	rat VEGF knockout BM-MSC	rat	P/E	↑ VEGF, HGF, IGF-1 ↓ mortality and ↑ functional recovery
Hara et al. [55]	rat BM-MSC	rat	P/E	↓ IL-1 β , TNF- α , IFN- γ inhibition of ICAM-1 ↓ macrophage infiltration (CD68+, CD3+, CD25+) ↓ DC attracting chemokines (CCL19, CCL21) ameliorates inflammation
Patel et al. [54]	rat GTSC	rat	P/E	↑ 2- to 8-fold of growth factors (FGF2, HGF, IGF-1, VEGF) and anti-inflammatory factors (IL-4, IL-10) abundance and easy accessibility compared to other SC

TGF- α = Transforming growth factor- α ; FGF2 = fibroblast growth factor 2; CD = cluster of differentiation; CCL = lymphoid chemokines; iNOS = inducible nitric oxide synthase.

Th2 profile [13]. In the rat model, improvement of renal function was followed by reduced expression of IL-6, IL-1 β , TNF- α and an increased expression of IL-4 and IL-10. The Bcl/Bcl-2-associated death promoter (Bcl-2/Bad) ratio was increased in treated animals at 24 h after ischaemia [13]. AKI associated with renal tubular death is mediated by apoptosis which is regulated by members of Bcl-2 family, and a decreased Bcl-2/Bad is favoured to cell death [13]. Hara et al. [55] showed that MSC application had a dose-dependent effect resulting in downregulation of proinflammatory cytokines (TNF- α , IFN- γ , IL-1 β). The inhibition of adhesion molecules (intercellular adhesion molecule 1, ICAM-1) resulted in a diminished macrophage infiltration (cluster of differentiation 68, CD68+), CD3+ T cells and activated CD25+ cells and reduced dendritic cells (DC-) attracting chemokines (lymphoid chemokines, CCL19 and CCL21), resulting in a diminished infiltration of DCs (Ox62+).

Combined Effect (Paracrine + Homing; table 2)

Wang et al. [56] in a rat model of IRI demonstrated that bone marrow mononuclear cells (BMMNC) led to a reduction of necrosis and apoptosis of renal tubular cells. This was due to an increase in cell proliferation due to differentiation of some transplanted BMMNC into renal tubular epithelial cells in addition to an increase in biological activity, due to release of various growth factors and trophic factors. Hagiwara et al. [57] similarly showed that using kallikrein-modified MSC (TK-MSC; combined SC and kallikrein gene approach) led to the migration and homing ability as well as paracrine effect, thereby enhanced renal protection and advanced benefits in healing the injured kidney. The expression of human tissue kallikrein in the rat kidney after TK-MSC implantation exhibited enhanced protection against renal injury by inhibiting apoptosis and inflammatory cell infiltration. Thus TK-MSC secreted recombinant human tissue kallikrein into culture medium and significantly improved stem cell survival during oxidative stress via activation of the phosphatidylinositol 3-kinases/protein kinase B (PI3K-Akt) pathway. At 48 h after IRI, TK-MSC inhibited interstitial neutrophil and monocyte/macrophage infiltration and decreased the myeloperoxidase activity, superoxide formation, p38 mitogen-activated protein kinase phosphorylation and expression of TNF- α , monocyte chemoattractant protein-1 and ICAM-1 [57]. Wang et al. [58] showed improved renal function when SC of neural origin (neural precursor cells, NPC) were administered in the rat IRI model. The NPC were exclusively found in renal parenchyma, and treatment resulted

in reduced proinflammatory IL-1 β and TNF- α expression and increased IL-4 and IL-10 transcription in addition to reduced macrophage infiltration. Similarly, Chen et al. [59] demonstrated that adipose-derived MSC (ADMSC) therapy minimised kidney injury after IRI by suppressing oxidative stress and inflammatory response. Furuichi et al. [12] also demonstrated a combined effect, where they were able to ameliorate induced AKI secondary to IRI with the use of ADMSC in a mouse model. The suppression of cytokines (IL-1 β and TNF- α) and chemokines (MIP-1 α) led to an anti-inflammatory activity and alleviation of tubular necrosis. In this model, surprisingly homing occurred mostly in the lung, and they suggested that cell therapy may play a role in disease process like sepsis where multiple organs are involved.

Patschan et al. [60] targeted endothelial progenitor cells (EPCs), which are cells that have been documented to participate in postnatal neovascularisation (vasculogenesis) [60–66]. In their murine model of IRI, they demonstrated that in vitro Epac-1 Ac (exchange protein directly activated by cAMP-1 activator) pre-activation of EPCs did not increase overall expression intensity but induced a redistribution of β_1 -integrins towards the cell membrane. They concluded that EPC pre-treatment with this integrin receptor activator augmented the anti-ischaemic potential of the cell and thus protected the kidney from acute IRI [60]. Huls et al. [67] demonstrated that either p-glycoprotein (P-GP) or breast cancer-resistant protein (BCRP) protected from AKI. In the mouse model of IRI, they demonstrated absence of renal damage when animals were transplanted with transporter-deficient bone marrow SC. They further concluded that bone marrow from P-GP- or BCRP-deficient mice contains significantly more monocytes and granulocytes as well as early outgrowth EPC which potentially fuel renal protection.

Stem cell plasticity is remarkable, and the broad plasticity is a possibility what determines the therapeutic potential for SC [68]. Wang et al. [58] suggested NPC treatment worked via influencing immunological responses, and describe that SC benefits are not tissue specific and thus show a direct link between neural molecules and renal protection. Orth et al. [69] have shown that growth and transcription factors similar to those essential to the nervous system operate in the kidney and are induced after an ischaemic episode. Shi et al. [70] support these findings. In their study, exogenous administration of glial cell-derived neurotrophic growth factor protected the kidney against IRI. Other research has shown neurospheres to produce growth factor [71] and cytokines [72–75], thus modifying tissue immune responses.

Table 2. Studies highlighting benefits of SC therapy in small animal models [combined paracrine/homing (P/H) and modified approaches (M)]

Reference	SC source	Animal	Mode of action	Features
Wang et al. [56]	BM-MNC mononuclear cells	rat	P/H	<ul style="list-style-type: none"> ↓ apoptosis and necrosis ↑ differentiation into tubular epithelial cells in renal parenchyma ↑ growth and trophic factors ↓ blood urea at 24 and 48 h
Shi et al. [70]	mouse kidney derived MSC expressing glial cell line-derived neurotropic factor	mouse	P/H	<ul style="list-style-type: none"> ↑ homing in renal cortex ↑ nestin expression ↓ oxidative stress ↑ VEGF ↑ renal function amelioration of IRI
Hagiwara et al. [57]	rat kallikrein-modified MSC	rat	P/H	<ul style="list-style-type: none"> ↓ apoptosis ↓ renal tubular injury ↓ serum creatinine and urea nitrogen ↓ tubular injury ↓ inflammatory cell infiltration
Wang et al. [58]	rat NPC	rat	P/H	<ul style="list-style-type: none"> homing of NPC in renal parenchyma ↓ IL-1β, TNF-α ↑ IL-10, IL-4 ↓ macrophage infiltration ↑ improved renal function
Patschan et al. [60]	murine EPC	mouse	P/H	<ul style="list-style-type: none"> preserved renal function (35-min ischemia) redistribution of β_1-integrin homing of EPC in renal medulla
Huls et al. [67]	mouse BM-MSC deficient in P-GP or BCRP protein	mouse	P/H	<ul style="list-style-type: none"> ↑ early outgrowth of EPC ↑ improved renal function (GFR and creatinine clearance) ↓ early inflammatory response 20–30% homing into renal parenchyma
Chen et al. [59]	rat ADMSC	rat	P/H	<ul style="list-style-type: none"> ↓ oxidative stress ↓ inflammatory response ↓ IL-1β, TNF-α, IFN-γ NFC-CFSE-positive cells homing in renal parenchyma ↑ IL-10, IL-4
Furuichi et al. [12]	mouse ADMSC	mouse	P/H	<ul style="list-style-type: none"> ↓ cytokines (IL-1β, TNF-α, IFN-γ) and chemokines (MIP-1α) ↓ inflammatory activity ↓ tubular necrosis distant homing in lung: possible benefit of use in clinical scenarios like sepsis associated with multiorgan failure
Nafar et al. [28]	mouse BM-MSC + GCSF	mouse	M	<ul style="list-style-type: none"> ↓ tubular necrosis ↑ renal function and histological recovery ↑ anti-apoptotic and anti-inflammatory factors ↑ angiogenic factor ↑ IL-10, hem-oxygenase-1, bone morphogenetic-7 ↓ IL-6, TGF-β, TNF-α
Tian et al. [96]	mouse BM-MSC + lipid mediator (14S,21R-diHDHA)	mouse	M	<ul style="list-style-type: none"> lipid mediator promotes MSC viability via PI3-AKT signalling pathway ↓ serum creatinine and cell infiltration ↓ apoptosis and renal tubular cell death ↓ TNF-α and reactive oxygen species ↑ HGF, IGF-1
Altun et al. [97]	rat BM-MSC + DPO	rat	M	<ul style="list-style-type: none"> ↓ serum creatinine and urea nitrogen ↑ histological recovery

Role of Resident Stem/Progenitor Cell Expansion and Proliferation

Following either ischaemic or toxic injury, the kidney has the potential to recover quickly [12]. This repair may be due to the surviving resident stem/progenitor cells undergoing epithelial cell spreading, cell de-differentiation and proliferation to cover the exposed area of the basement cell membrane [12]. The participation of integrins and cell adhesion molecules leads to repair; they are up-regulated by stem cell therapy and in themselves represent regulators of migration, differentiation and proliferation. For example, release of HGF and IGF-1 can potentiate recovery of surviving cells and de-differentiation of tubule cells and their proliferation [12, 14, 76]. In addition, expression of differentiation factors like neural cell adhesion molecule and osteopontin leads tubular cells to re-differentiate and repolarise [12]. Administration of exogenous MSC led to alteration of all the IRI responses described above. Several studies have shown amelioration of various functional parameters [13, 23, 30, 77–83], and this is thought to be due to the paracrine/endocrine effects of MSC leading to an acceleration of regeneration of proximal tubular cells [30, 79, 84]. There is little evidence to support the hypothesis of transdifferentiation [30, 85, 86]. Recently Angelotti et al. demonstrated that CD133+CD24+ renal progenitors can differentiate into podocyte progenitors or tubular progenitors. These tubular committed progenitors display resistance to death, higher proliferation potential than other differentiated renal cells and regenerate into tubular cells following AKI. They concluded that the study provided further evidence of human renopoeitic system and novel treatments could be aimed at promoting the regeneration capacity of these cells to prevent and treat tubular injury [87].

To add a further level of complexity, Nafar et al. [28] in a rat model of IRI administered MSC in addition to granulocyte colony-stimulating factor (G-CSF), and were able to attenuate the recovery course of IRI as well as ameliorate tubular necrosis. They showed that G-CSF works as a bone marrow stem cell mobilising cytokine which facilitates renal tubular regeneration; similar results were produced by Fang et al. [88]. Nafar et al. [28] evaluated these effects by measuring CD34+ cells in peripheral blood via flow cytometry, and demonstrated increased peripheral blood counts of the CD34+ cell lineage, thus suggesting that mobilisation of endogenous BMSCs may be an additional factor to SC playing a direct structural role or inserting paracrine/endocrine effects. Feng et al. [89] in a rat IRI model showed that administration of

freshly isolated, uncultured, adipose tissue-derived SC (ADRC) significantly reduced mortality (survival: 100 vs. 5%; ADRC vs. control, respectively) and improved renal function (serum creatinine: 3.03 ± 1.58 vs. 7.32 ± 2.32 ; ADRC vs. control, respectively). In addition, they demonstrated a significant downregulation of the proinflammatory cytokines like chemokine (C-X-C motif) ligand 2 (CXCL2) and IL-6 (mRNA expression at 24 h following AKI). They demonstrated that ADRC enhance tubular cell proliferation as detected by an increase in ki-67-positive cells as early as 24 h following stem cell infusion, though the ADRC cells were found predominantly in the glomeruli and not the tubules.

Similarly, Kwon et al. [90] showed that recipient bone marrow-derived EPC (CD34+ EPC) and endothelial cells (CD146-positive EC) decreased in the circulation immediately following IRI. These cells were at this particular time point observed along the endothelial lining in peritubular capillaries in the ischaemic damaged kidney, and were not seen in their counterpart non-ischaemic controls. They suggested that the tissue regeneration maybe the result of proliferating surviving de-differentiated cells and renal SC, findings supported by other studies [78, 79, 91]. Kim et al. [92] concluded that intra-renal rodent cells have stem- or progenitor-like characteristics which contribute to the restoration of damaged tubular epithelial cells. Using 5-bromo-2'-deoxyuridine (BrdU)-labelled slow cell cycle cells, they observed that BrdU-labelled cells were mainly located at either the junction of the cortex and outer medulla or the inner medulla. These BrdU-labelled cells not only survived IRI but thereafter proliferated and expressed functional tubule cell markers such as Na/K-ATPase, Na-K-Cl co-transporter-2 and aquaporin 1 and 2. The beneficial effects of SC are thus further enhanced by generating a favourable environment created by administered SC where the resident stem/progenitor cell expansion and proliferation can take place and thus contribute to reduced injury [93–95].

Modified Methods (SC and Additive Pharmacological Agents; table 2)

Recently Tian et al. [96] developed a modified stem cell method, where they have shown that MSC enhanced by treatment with lipid mediator [14S,21R-dihydroxydocosahexaenoic acid (14S,21R-diHDHA)] further ameliorated IRI. The mice subjected to treated MSC showed reduced serum creatinine levels, reduced tubular cell death, inhibition of neutrophils, macrophages and DC infiltration within the kidney in addition to reduced TNF- α and reactive oxygen species levels. Treatment with 14S,21R-

Table 3. Studies highlighting studies of SC therapy in large animal models

Reference	SC source	Effect	Animal	Mode of action	Features
Zhen-Qiang et al. [98]	rabbit BM-MSC	positive	rabbit	Combined paracrine/homing (P/H)	↑ Bcl-2, Bcl-2-associated protein levels ↑ superoxide dismutase activity ↑ tissue regeneration in kidney renal tubule homing of MSC ↑ recovery of renal function ↓ TNF- α , TGF- β , IL-1 β
Behr et al. [106]	ovine autologous BM-MSC	negative	sheep	–	no reparative or paracrine properties
Chade et al. [99]	porcine autologous EPC	positive	pig	P/H	↑ stromal-derived factor-1, angiopoietin-1, Tie-2, c-kit expression and integrin- β_2 ↑ and restored angiogenic activity, improved microvascular density ↑ renal hemodynamics and function ↓ fibrosis ↓ oxidative stress
Eirin et al. [100]	porcine allogenic ADMSC	positive	pig	P/H	↑ renal function ↑ and restored hemodynamics ↓ inflammation ↓ apoptosis ↓ oxidative stress ↓ microvascular loss and fibrosis
Brunswick-Spickenheier et al. [108]	porcine allogeneic MSC	negative	pig	P/H	inadequate immune-modulating effects failed to inhibit the mixed lymphocyte reaction ↑ IL-6

diHDHA thus promoted the viability of MSC in vivo through mechanism involving activation of PI3K/AKT signalling pathway and promoted secretion of renotrophic HGF and IGF-1, thereby rendering the cells resistant to apoptosis. Nafar et al. [28] administered MSC in addition to GCSF and were able to attenuate tubular necrosis. Altun et al. [97] in a rat model demonstrated that treatment with MSC and darbepoetin- α (DPO) concomitantly improved renal function when compared with the controls. Histological analysis demonstrated that tissue injury was significantly decreased in rats treated concomitantly with MSCs and DPO. These results suggest that concomitant application of SC and other pharmacological agents may be the potential novel renoprotective therapy for patients who have sustained an ischaemic renal insult.

Large Animal Models and Role of Potential Stem Cell Benefits (table 3)

Giraud et al. [1] recently published large animal model work. They showed benefits of porcine model over small animal models; the porcine model is a species

which is characterised by human-like renal anatomic structure, multiparity and multilobular kidney. In addition, they can be used to create models which realistically simulate day-to-day practice human scenarios like DCD transplantation. Rodent models, though less expensive, have specific use and data extrapolation to a human situation is limited by anatomic and physiological differences. Zhen-Qiang et al. [98] published the beneficial effects of SC in a larger animal model using rabbits. Rabbits injected immediately after I/R with human bone morphogenic protein-7-transduced BM-MSCs showed a higher activity of superoxide dismutase, and a lower amount of malondialdehyde. The increased Bcl-2 and Bcl-2-associated X protein levels indicated the regeneration of the kidney in the experimental group. Chade et al. [99] in an atherosclerotic renal artery stenosis (ARAS) model showed that pigs injected with autologous EPCs showed increased stromal derived factor-1, angiopoietin-1, Tie-2 and c-kit expression. In addition, the ARAS kidney itself releases c-kit-ligand stem cell factor and upregulated integrin- β_2 , suggesting activation of cor-

responding homing signals. This relates to the injured kidney as to restored angiogenic activity, improved microvascular density, renal hemodynamics, function, decreased fibrosis and oxidative stress and attenuated endogenous injury signals.

Eirin et al. [100] showed that a single intrarenal delivery of labelled allogenic adipose tissue-derived MSC (isolated from a normal swine) into a pig subjected to ARAS restored the haemodynamics, renal function and reduced inflammation, apoptosis, oxidative stress, microvascular loss and fibrosis. These findings thus shed light not only onto the renoprotective mechanism (chronic injury models [99, 100]), but also feasibility. The potential of such autologous and allogenic cell-based therapy can also be applied to scenarios of AKI (similar mechanisms), such as transplantation (where injury is anticipated). Further larger animal model studies are thus required to assess the effects of SC in renal ischaemia injury not only to assess efficiency but also safety, prior to translating research towards humans.

Evidence Showing No Benefits of SC in Renal IRI

Weissman et al. [101] demonstrated very little plasticity of HSC in mice following IRI, and concluded that differentiation of HSC to non-HSC does not occur in every animal model. Jiang et al. [102] in a mice model concluded that transplantation using HSCs or MSCs cell line did not improve the renal repair process. Similarly, Huls et al. [103] demonstrated that following IRI, approximately 10% of integrated tubular segments originated from transplanted bone marrow cells, but also showed that a significant decrease in creatinine clearance was observed at day 2 following ischaemia. This improved to normal levels on day 7 after ischaemia, similar to IRI damaged controls not receiving cell therapy. A mouse model developed by da Silva et al. [104] used BMSCs with or without FTY720 (a compound promoting preferential migration of lymphocytes to lymph nodes rather than to an inflammatory site), which did not demonstrate early kidney recovery following IRI. Similar findings were reported by Kale et al. [30] showing that injected BMSCs were not found in the kidney up to 3 weeks after administration, but 12 weeks later tubules contained 2–5% BMSCs. This paper unfortunately did not quantify which bone marrow-derived SC were present (recipient or donor). No improvement was seen in renal function, and the authors concluded that the majority of tubule repair occurs probably via proliferation of endogenous renal cells rather

than incorporation of BMSCs or by the protective factors secreted by these cells, findings later demonstrated by Lin et al. [93].

Duffield et al. [80] in a mouse model using an intravenous injection of bone marrow mesenchymal stromal cells demonstrated no evidence of differentiation into tubular cells indicating that BMSC do not contribute to restoration of epithelial integrity after an ischaemic insult. This was measured with cells expressing GFP, bacterial β -gal or harbouring male chromosome exclusively in these bone marrow-derived cells. This group also concluded tubule regeneration occurs by survival of de-differentiated epithelial cells which proliferate and re-differentiate into mature epithelial cells, as SC of bone marrow origin do not differentiate into renal cells [105]. De Broe [85] concluded that renal repair after acute ischaemic toxic injury seems to be limited to the kidney itself. Mechanism include the mobilisation of growth factors, stimulation of surviving de-differentiated cells, upregulation of protective proteins and recruiting SC localised deep in the kidney. They caution against the premature clinical use of bone marrow cells in treatment of renal failure and suggest that HSC mobilisation-associated granulocytosis may worsen renal failure.

Referring back to Giraud et al. [1] and advantages of large animal models, Behr et al. [106] published their results of an autologous large animal model (ovine). They summarised that the model was a relevant large animal model of kidney IRI providing a reliable setting for evaluation of MSC therapy. The MSC treatment did not exhibit reparative or paracrine properties (TNF- α , VEGF- α , Bcl-2, caspase-1), and no improvement was seen in renal function following 60 min of ischaemic damage. They concluded that there may be fundamental differences in species and stemness biology (stem cell turnover is remarkably higher in small animals than larger ones) [107], which make translation of results from rodent models to humans hazardous. Brunswig-Spickenheier et al. [108] in a porcine model contrary to their rat model demonstrated that porcine MSCs failed to inhibit the mixed lymphocyte reaction and induced robust production of proinflammatory IL-6. The authors concluded that MSCs exert inadequate immune-modulating effects and are not kidney protective (table 3).

Potential Risk of Stem Cell Therapy

Reinders et al. [22] highlight the possible risk associated with the use of SC therapy. For example, a patient treated with HSCs developed renal lesions which on ne-

phrectomy were confirmed to be angiomyeloproliferative lesions, which were stem cell (HSC) derived or stem cell (HSC) induced [108]. The other concern they raised was lack of standardisation of these fresh cell products. The use of ESC (embryonic SC) is associated with a risk of malignant transformation and teratoma formation as seen with induced pluripotent stem (iPS) cells. iPS cells express Sox2 and c-myc, and c-myc is an oncogene which may contribute to tumour genesis by overstimulation of cell growth or by causing genomic instability [109, 110]. Reassuringly, Lee et al. [111] in rat model demonstrated improved renal function and survival and no evidence of tumour formation at 6 months, though 6 months may be a too short follow-up period. The risk of thrombosis and embolisation exists, and can worsen ischaemia and lead to potential end organ damage following intra-arterial administration of SC [112].

Human Clinical Studies in AKI

Patschan et al. [113] reported that sepsis is associated with significant impairment of EPC system. They showed increased EPC numbers, increased concentrations of proangiogenic mediators and reduced proliferation capacity of cells. In addition, the cohort of patients with acute renal dysfunction showed an even more pronounced elevation of CD133+/Flk+ cells, though average proangiogenic cytokines were not proportionally raised. Thus, acute renal dysfunction possibly activates 'vascular danger signals' in order to activate endogenous repair mechanisms, suggesting possible role of stem cell therapy with EPCs may improve the outcome in patients with sepsis and associated renal failure, in line with other studies [44, 112–114].

Westenfelder and Togel [115] address the role of allogeneic MSC in preventing and treating AKI following on-pump coronary artery bypass graft or cardiac valve surgery with a suprarenal aortic infusion. They showed it to be safe and feasible; in addition, the MSC therapy prevented postoperative deterioration in renal function (20% AKI incidence in case control), reduced the length of stay (40%) and need for readmission (40%) when compared to well-matched historical case controls [116–119]. The authors plan a double-blind, controlled, multicentre phase II efficacy study once late safety data are documented. They concluded that off-the-shelf use of allogeneic MSC should thus be possible and safe as the MSC are hypoimmunogenic [do not express HLA-II (DR) and blood group antigens] [25, 115, 117, 118]. They remain in the acutely

injured kidney (and other organs) for a very short period of time, work via their paracrine and endocrine (VEGF, HGF, IGF-1) [25, 115, 117–121] modes of action and lack ectopic differentiation and oncogenic transformation.

Other documented phase I trials in the AKI situation include administration of MSC to kidney transplant recipients from living unrelated donors [122], patients with chronic allograft nephropathy, lupus nephritis currently conducted in China (Fuzhou Hospital) and for treatment of kidney transplant rejection in the Netherlands (Leiden University) (<http://www.clinicaltrials.com>) [116]. Tan et al. [122] in a randomised controlled trial demonstrated that in patients undergoing a live related renal transplant, the use of autologous MSC compared with anti-IL-2 receptor antibody induction therapy resulted in lower incidence of acute rejection, decreased risk of opportunistic infection, and better estimated renal function at 1 year. Patient and graft survival at 13–30 months was similar. This study unfortunately did not address the underlying mechanism, although short-term safety data are promising. Published data in a large animal model and human setting (safety, short-term and long-term) are still lacking.

Conclusions

Although the majority of studies in the field of renal IRI and stem cell therapy show remarkable benefits, they are mostly confined to small animal models. These results should be taken with caution and first translated to a large animal model such as the pig. Controversy exists regarding the exact mechanism, and thorough scientific exploration is required to assess mechanism, safety profile, reproducibility and methods to monitor administered SC.

Owing to the high morbidity and mortality associated with IRI/AKI and disappointing results from current available clinical therapeutic approaches, stem cell therapy may be promising, but it is imperative not to create unrealistic goals and possibly translate danger to patients.

Disclosure Statement

The authors have no conflicts of interest to disclose as described by the *American Journal of Nephrology*.

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